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AAAAATAAATCAT ATG AAA AAT ATT AAA AAA AAT CAA GTA ATC AAT CTC GGT CCT AAT TCT  
 M K N I K K N Q V M N L G P N S  
  
 AAA TTA TTA AAA GAA TAT AAA TCA CAA TTA ATT GAA TTA AAT ATT GAA CAA TTT GAA GCA  
 K L L K E Y K S Q L I E L N I E Q F E A  
  
 GGT ATT GGT TTA ATT TTA GGA GAT GCT TAT ATT CGT AGT CGT GAT GAA GGT AAA ACT TAT  
 G I G L I L G D A Y I R S R D E G K T Y  
  
 TGT ATG CAA TTT CAC TCC AAA AAT AAG GCA TAC ATG GAT CAT GTA TGT TTA TTA TAT GAT  
 C M Q F E W K N K A Y M D H V C L L Y D  
  
 CAA TGG GTA TTA TCA CCT CCT CAT AAA AAA GAA AGA GTT AAT CAT TTA GGT AAT TTA GTA  
 Q W V L TCA CCT CCT CAT AAA AAA GAA AGA GTT AAT CAT TTA GGT AAT TTA GTA  
  
 ATT ACC TGG GGA GCT CAA ACT TTT AAA CAT CAA GCT TTT AAT AAA TTA GCT AAC TTA TTT  
 I T W G A Q T F K H Q A F N K L A N L F  
  
 ATT GTA AAT AAT AAA CTT ATT CCT AAT AAT TTA GTT GAA AAT TAT TTA ACA CCT ATG  
 I Y N N K K L I P N N L V E N Y L T P M  
  
 AGT CTG GCA TAT TGG TTT ATG GAT GAT GGA GGT AAA TGG GAT TAT AAT AAA AAT TCT CTT  
 S L A Y W F M D D G G K W D Y N K N S L  
  
 AAT AAA AGT ATT GTA TTA AAT ACA CAA AGT TTT ACT TTT GAA GAA GTA GAA TAT TTA CTT  
 N K S I I V L N T Q S F T F E V C Y L V  
  
 AAA GGT TTA AGA AAT AAA TTT CAA TTA AAT TGT TAT GTT AAA ATT AAT AAA AAT AAA CCA  
 K G L R N K F Q L N C Y V K I N K N K P  
  
 ATT ATT TAT ATT GAT TCT AGT AGT TAT CTG ATT TTT TAT AAT TTA ATT AAA CCT TAT TTA  
 I I Y I D S M S Y L I F Y N I T K P Y L  
  
 ATT CCT CAA ATG ATG TAT AAA CTG CCT AAT ACT ATT TCA TCC GAA ACT TTT TTA AAA TAA  
 I P Q M Y K L P N T I S S E T F L K

FIG. 1

1 and 2:

THESE AMINO ACIDS ARE ABSOLUTELY NECESSARY TO PRODUCE CATALYTIC ACTIVITY. OTHER SUBSTITUTIONS ARE POSSIBLE, SUCH AS DELETIONS OF THE 10 FIRST AMINO ACIDS.

FIG. 2

FIG. 3

1667 GCGGACAGGTATCCGGTAAGCGGCGAGGTCGGAACAGGAGAGCGCACGAGGGGAGCTTCCAGGGGGAAACGCCCTGGTATCT 1746  
1747 TTATAGTCCTGTCGGGTTTCGCCACCTCTGACTTGAGCGTCGATTTTGTG ATG CTC GTC AGG GGG GCG GAG 1818  
1819 CCT ATG GAA AAA CGC CAG CAA CGC GGC CTT TTT ACG GTT CCT GGC CTT TTG CTG GCC TTT 1878  
1879 TGC TCA CAT GTT CTT TCC TGC GTT ATC CCC TGA TTCGTGGATAACCGTATTACCGCCTTTGAGTGAGC 1947  
1948 TGATACCGCTGCGCGCAGCCGAACGACGAGCGCAGCGAGTCAGTGAGCGAGGAAGCGGCAATACGCAAC 2027  
2028 CGCCTCTCCCCGCGGTTGGCCGATTTCATTA ATG CAG CTG GCA CGA CAG GTT TCC CGA CTG GAA AGC 2094  
2095 GGG CAG TGA GCGCAACGCAATTA ATG TGA GTTAGCTCACTCATTAGGCACCCCGGCTTTACACTTT ATG 2164  
2165 CTT CCG GCT CGT ATG TTG TGT GGA ATT GTG AGC GGA TAA CAATTTACACAGGAACAGCT ATG 2228  
2229 ACC ATG ATT ACG AAT TCT CAT GTT TGA CAGCTTATCATCGATAAGCTTTA ATG CCG TAG TTTATCAC 2295  
2296 AGTTAAATTGCTAACGCAGTCAGGCACCGTGT ATG AAA TCT AAC AAT GCG CTC ATC GTC ATC CTC GGC 2363  
2364 ACC GTC ACC CTG GAT GCT GTA GGC ATA GGC TTG GTT ATG CCG GTA CTG CCG GGC CTC TTG 2423  
2424 CCG GAT ATC CGC CTG ATG CGT GAA CGT GAC GGA CGT AAC CAC CGC GAC ATG TGT GTG CTG 2483  
2484 TTC CGC TGG GCA TGC CAG GAC AAC TTC TGG TCC GGT AAC GTG CTG AGC CCG GCC AAG CTT 2543

FIG. 4A

2544	ACT	CCC	CAT	CCC	CCT	GTT	GAC	AAT	IAA	TCATCGGCTCGTATA	ATG	IGT	GGG	ATT	GTG	AGC	GGG	2606
73	T	P	H	P	P	V	D	N			M	C	G	I	V	S	G	7
2607	TAA	CAATTCACACAGGAACAGGATCC	BamHI															
8																		
2671	AAC	CTG	GGT	CCG	AAC	TCT	AAA	CTG	CTG	AAA	GAA	TAC	AAA	TCC	CAG	CTG	ATC	2670
13	N	L	G	P	P	N	S	K	L	K	E	Y	K	S	Q	L	I	12
2731	ATC	GAA	CAG	ITC	GAA	GCA	GGT	ATC	CTG	ATC	CTG	ATC	CTG	ATC	GAA	CTG	AAC	2730
33	I	E	Q	F	E	A	G	I	G	L	I	L	G	D	A	Y	I	32
2791	GAT	GAA	GGT	AAA	ACC	TAC	TGT	ATG	CAG	ITC	GAG	TGG	AAA	AAC	AAA	GCA	TAC	2790
53	D	E	G	K	T	Y	C	M	Q	F	E	W	K	N	K	A	Y	52
2851	GTA	CTG	CTG	TAC	GAT	CAG	TGG	GTA	CTG	TCC	CCG	CCG	CAC	AAA	AAA	GAA	CGT	2850
73	V	C	L	L	Y	D	Q	W	V	L	S	P	H	K	K	E	R	72
2911	CAC	CTG	GGT	AAC	CTG	GTA	ATC	ACC	TGG	GGC	GCC	CAG	ACT	ITC	AAA	CAC	CAA	2910
93	H	L	G	N	L	V	I	T	W	G	A	Q	T	F	K	H	Q	92
2971	AAA	CTG	GCT	AAC	CTG	TTC	ATC	GTT	AAC	AAC	AAA	AAA	ACC	ATC	CCG	AAC	AAC	2970
113	K	L	A	N	L	F	I	V	N	N	K	K	T	I	P	N	N	112
3031	AAC	TAC	CTG	ACC	CCG	ATG	TCT	CTG	GCA	TAC	TGG	ITC	ATG	GAT	GAT	GGT	AAA	3030
133	N	Y	L	T	P	M	S	L	A	Y	W	F	M	D	D	G	K	132
3091	TAC	AAC	AAA	AAC	TCT	ACC	AAC	AAA	TCG	ATC	GTA	CTG	AAC	ACC	CAG	TCT	ITC	3090
153	Y	N	K	N	S	T	N	K	S	I	V	L	N	T	Q	S	F	152
3151	GAA	GTA	GAA	TAC	CTG	GTT	AAG	GGT	CTG	CGT	AAC	AAA	ITC	CAA	CTG	AAC	TGT	3150
173	E	V	E	Y	L	V	K	G	L	R	N	K	F	Q	L	N	C	172
3211	ATC	AAC	AAA	AAC	AAA	CCG	ATC	ATC	TAC	ATC	GAT	TCT	ATG	TCT	TAC	CTG	ATC	3210
193	I	N	K	N	K	P	I	I	Y	I	D	S	M	S	Y	L	I	192
3271	CTG	ATC	AAA	CCG	TAC	CTG	ATC	CCG	CAG	ATG	ATG	TAC	AAA	CTG	CCG	AAC	ACT	3270
213	L	I	K	P	Y	L	I	P	Q	M	M	Y	K	L	P	N	T	212
3331	GAA	ACT	TTC	CTG	AAA	TAA												3330
233	E	T	F	L	L	K												232
3404																		3404
238																		238

FIG. 4B

~~Positions that can be changed without affecting enzyme activity (demonstrated positions -1 and -2 are not natural. The two amino acids are added due to cloning strategies)~~

### Changes that affect enzyme activity (demonstrated)

FIG. 5

Group I Intron Encoded Endonucleases and Related Endonucleases

ENDONUCLEASE		RECOGNITION SEQUENCE	CLEAVAGE SITE	▽ INTRON SITE
TWO DODECAPEPTIDE FAMILY (OR 4 BP CUTTERS)	I-Sce I (Saccharomyces mitochondria)	CGCTAGGGATAACAGGGTAATATAGC CGCATCCCTATTGTCCATTATATCG		
	I-Sce IV (Saccharomyces mitochondria)	TTCTCATGATTAGCTCTAATCCATGG AAGAGTACTAATCGAGATTAGGTACC		
	I-Sce II (Saccharomyces mitochondria)	CTTTGGTCAICGAGAAGTATATATT GAAACCAGTAGGTCTTCATATATAAA		
	I-Ceu I (Chlamydomonas chloroplast)	TAA CGGTCCIAAGGTAGCGAAATTCA ATTGCCAGGATTCCATCGCTTTAAGT		
	I-Ppo I (Physarum nucleus)	TGACTCTCTIAAGGTAGCCAAATGCC ACTGAGAGAATTCATCGGTTTACGG		
	I-Sce III (Saccharomyces mitochondria)	GGAGGTTTTGGTAACTATTTATTACC CCTCCAAAACCATGATAAATAATGG		
	I-Cre I (Chlamydomonas chloroplast)	GGGTTCAAAACGTCGTGAGACAGTTT CCCAAGTTTTGCAGCACTCTGTCAA		
	Endo. Sce I(RF3) (Saccharomyces mitochondria) (Non intronic)	GATGCTGTAGGCATAGGCTTGCTTAT CTACGACATCCGTATCCGAACCAATA		
	HO (Saccharomyces nucleus) (Non intronic)	CTTTCCGCAACAGTATAATTTTATAA GAAAGCGTTGTCAATATTAAAAATATT		
	I-Csm I (Chlamydomonas mitochondria) (Putative endonuclease)	ACCATGGGGTCAAATGTCTTTCTGGG TGGTACCCCGATTTACAGAAAGACCC		
	I-Pan I (Podospira mitochondria) (Putative endonuclease)	GTGCCTGAATGATATTIATTACCTTT CACGGACTTACTATAAATAATGGAAA		
OTHER STRUCTURAL FAMILIES	(Bacteriophage T4)			
	I Tev I	CAACGCTCAGTAGATGTTTTCTTGGGTCTACCGTTTAAAT GTTCGAGTCATCTACAAAAGAACCCAGATGGCAAATTA		
	I Tev II	CAAGCTTATGAGTATGAAGTGAACACGTTATT GTTGCAATACTCATACTTCACTTGTGCAATAA		
	I Tev III	GCTATTGTTTTTATGTATCTTTTGGCTGTAGCTTTAA CGATAAGCAAAAATACATAGAAAACGCACATCGAAATT		

FIG. 6



# EXPRESSION VECTORS

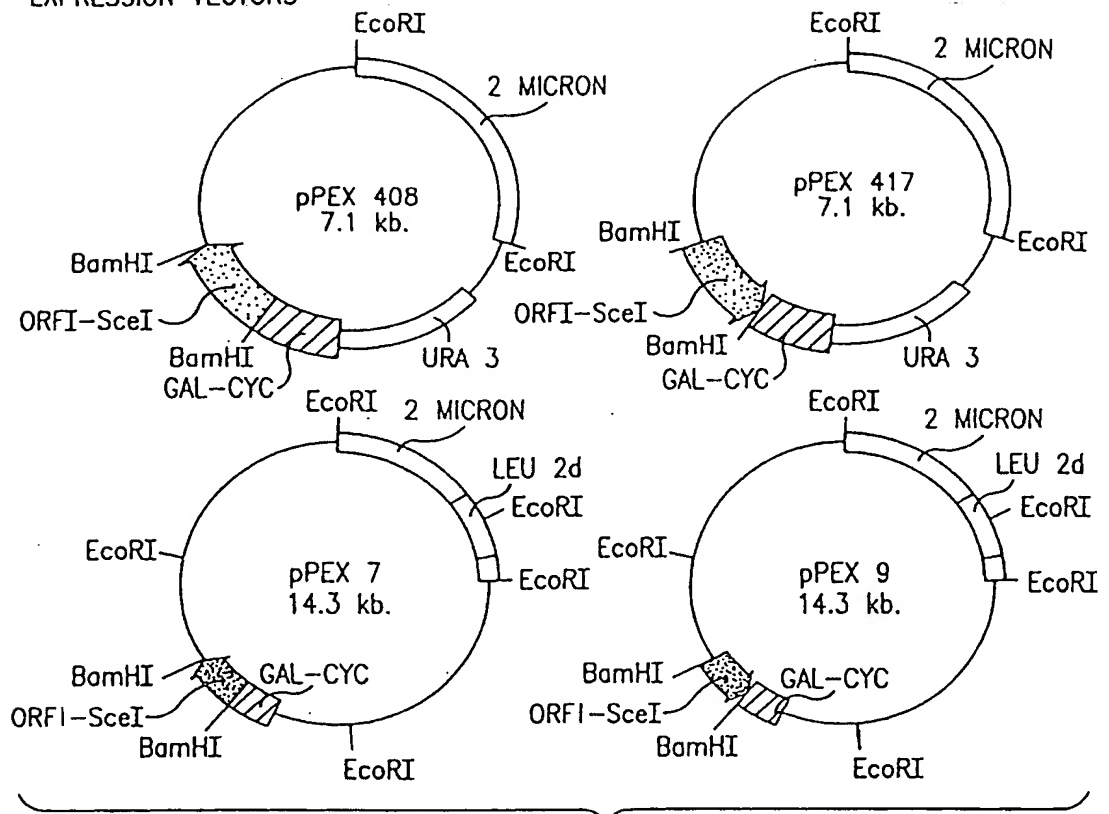


FIG. 7

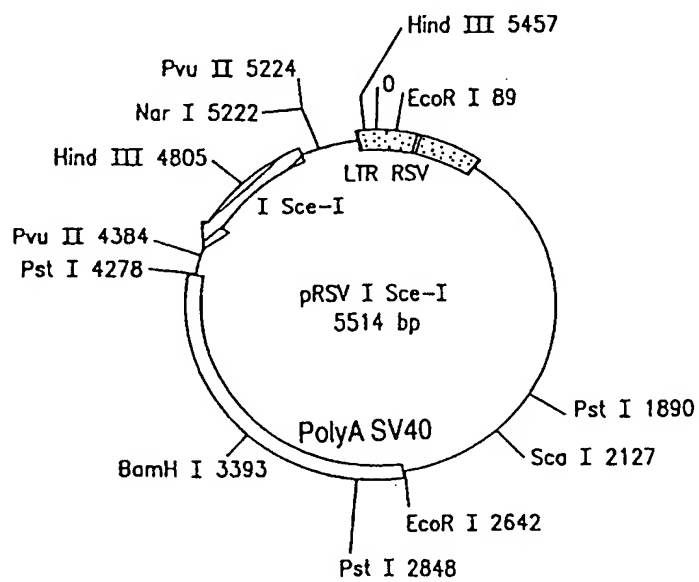


FIG. 8

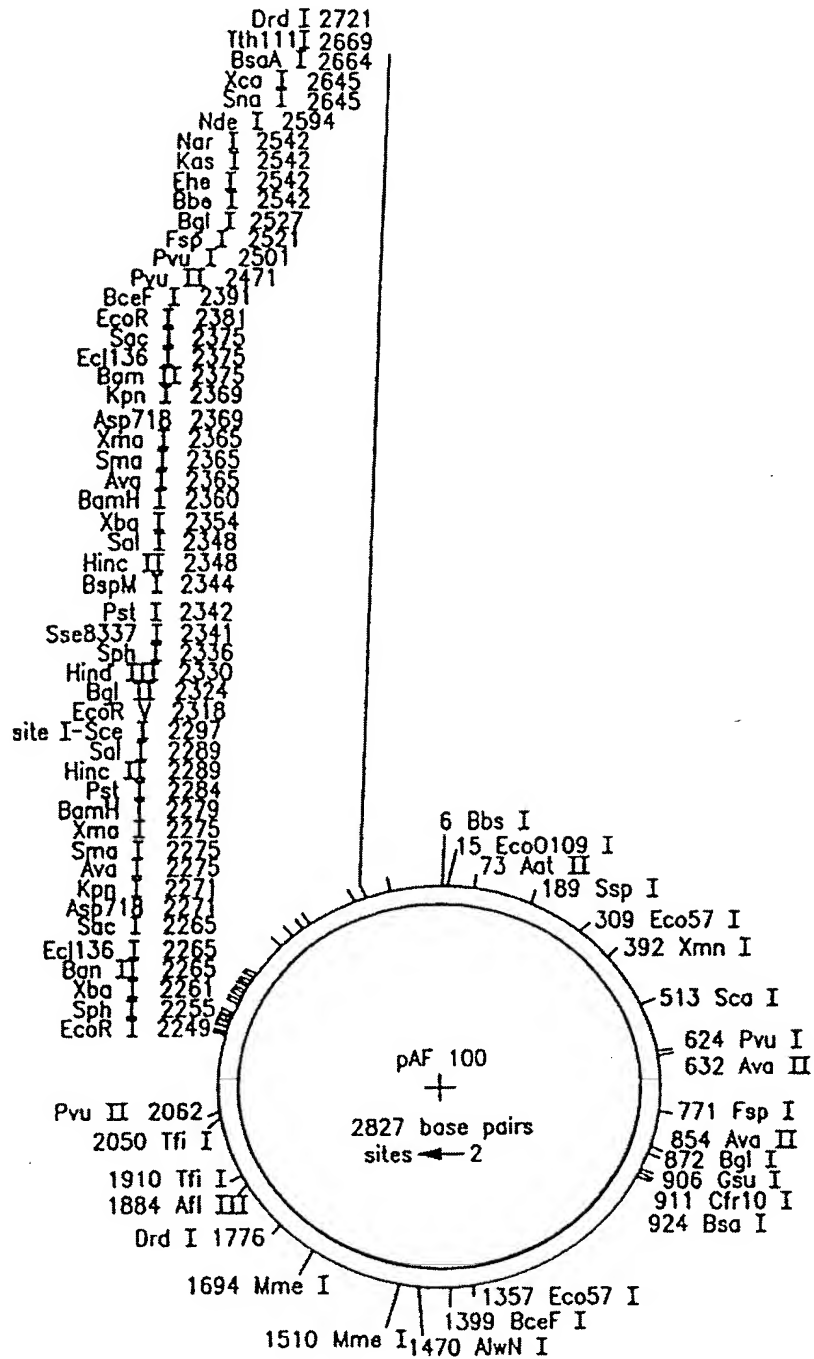


FIG. 9

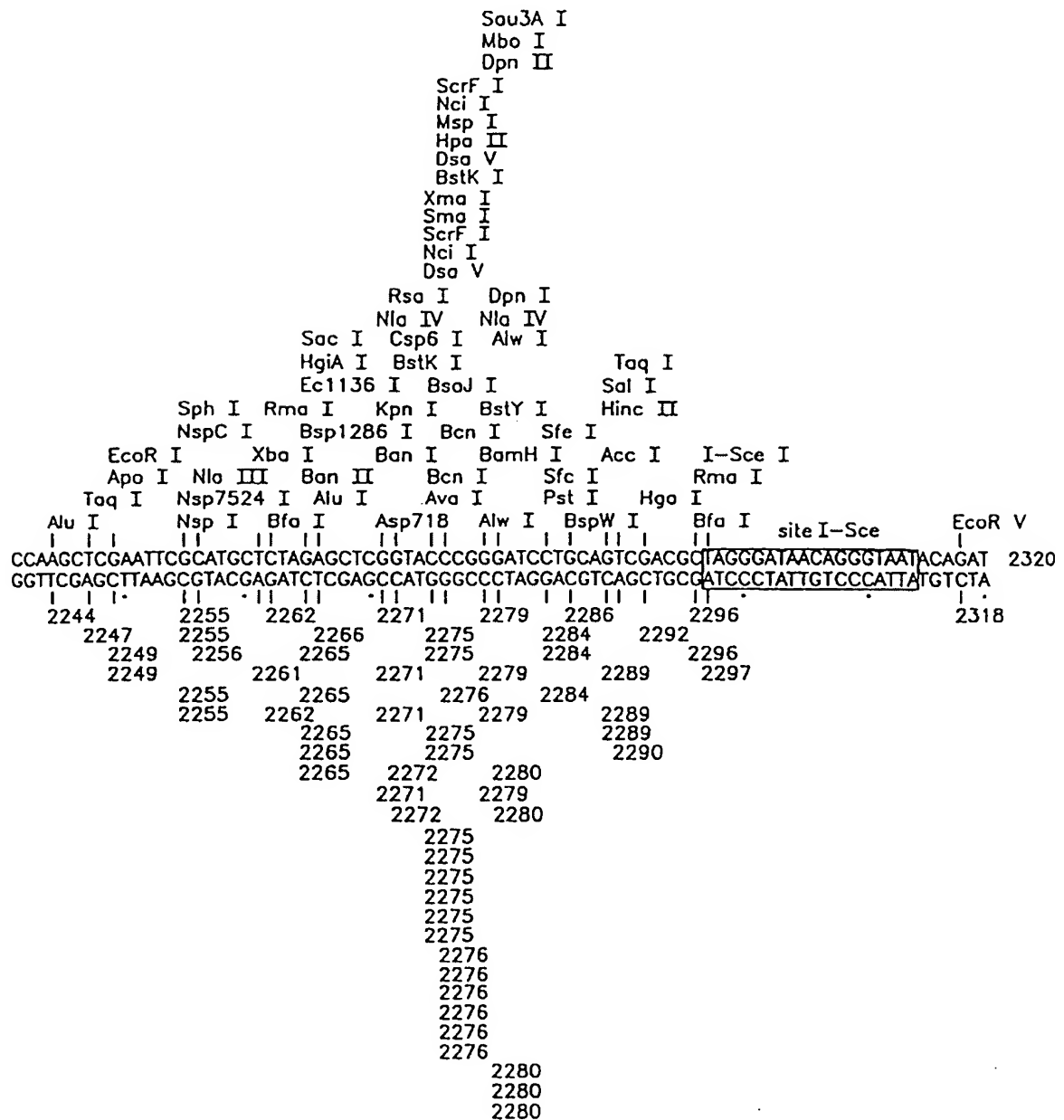
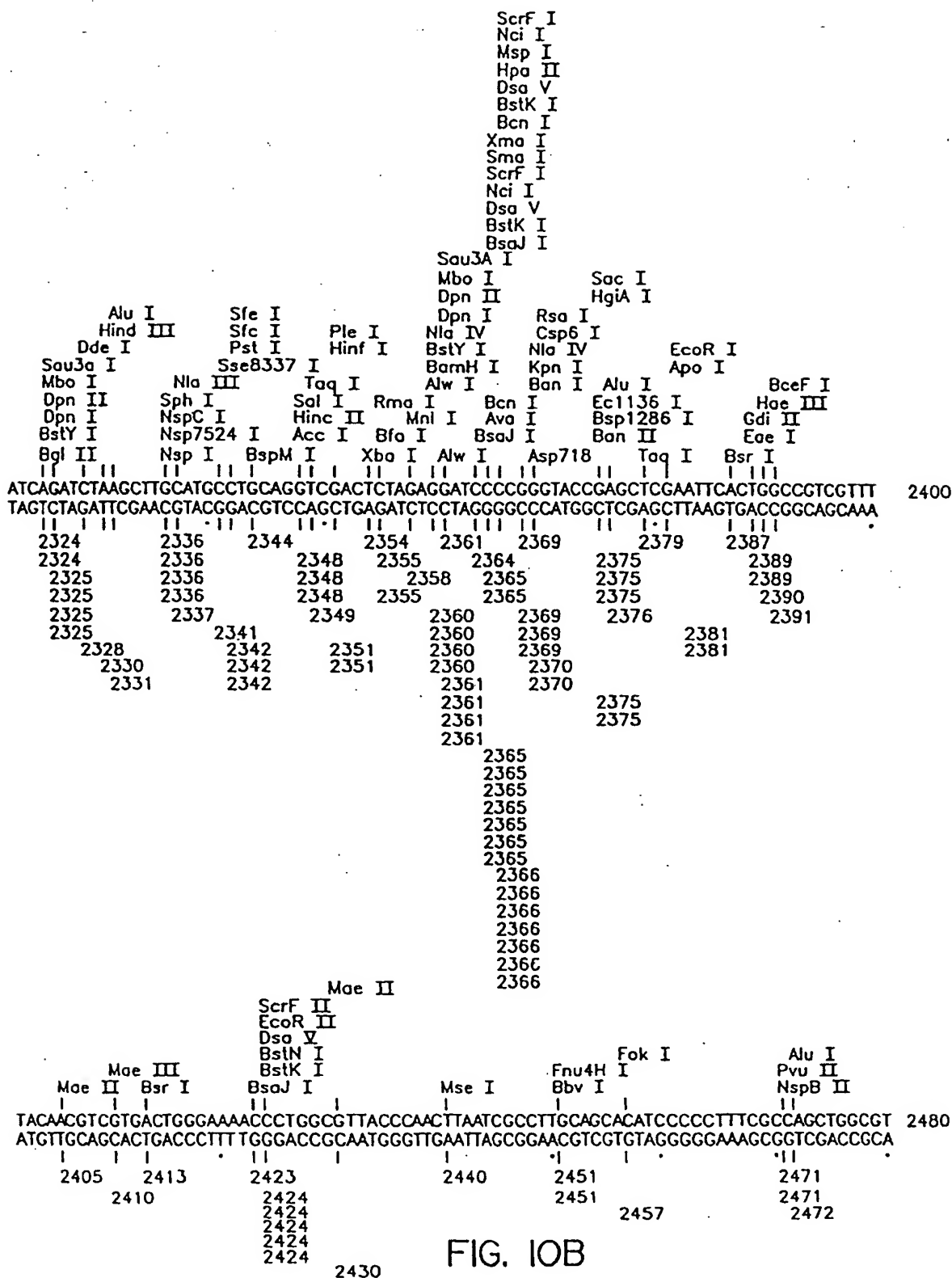
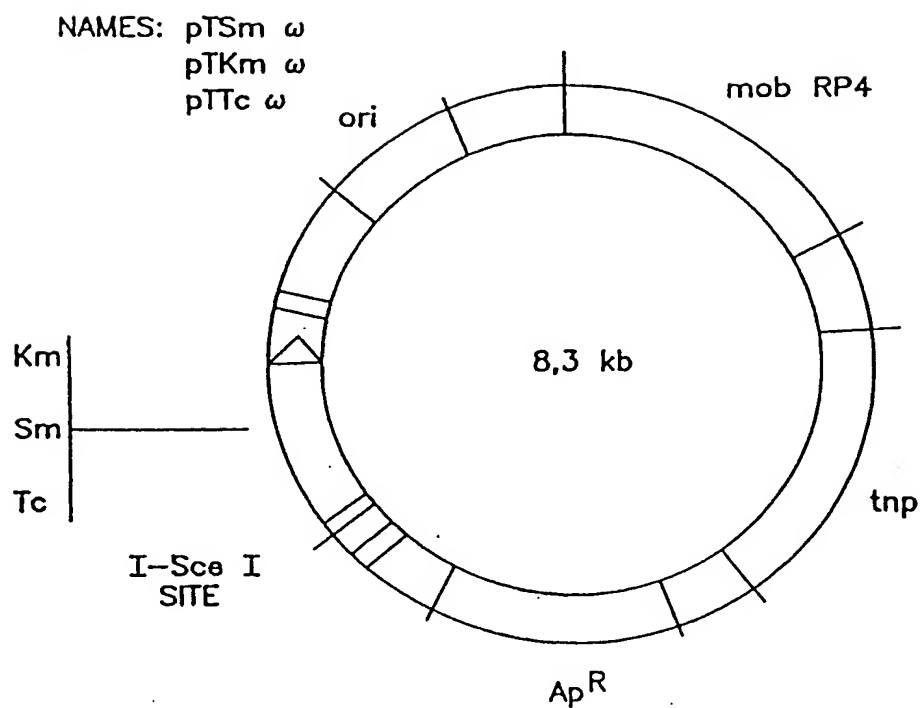


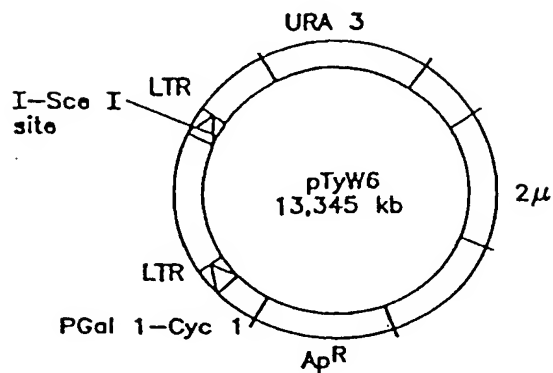
FIG. 10A





Construction: pGP 704 from De Lorenzo, with transposase gene and insertion of the linker[I-SceI] in NotI unique site

FIG. 11



Construction: pD 123, from J.D. Boeke  
with insertion of a linker[I-SceI-NotI] in BamHI

FIG. 12

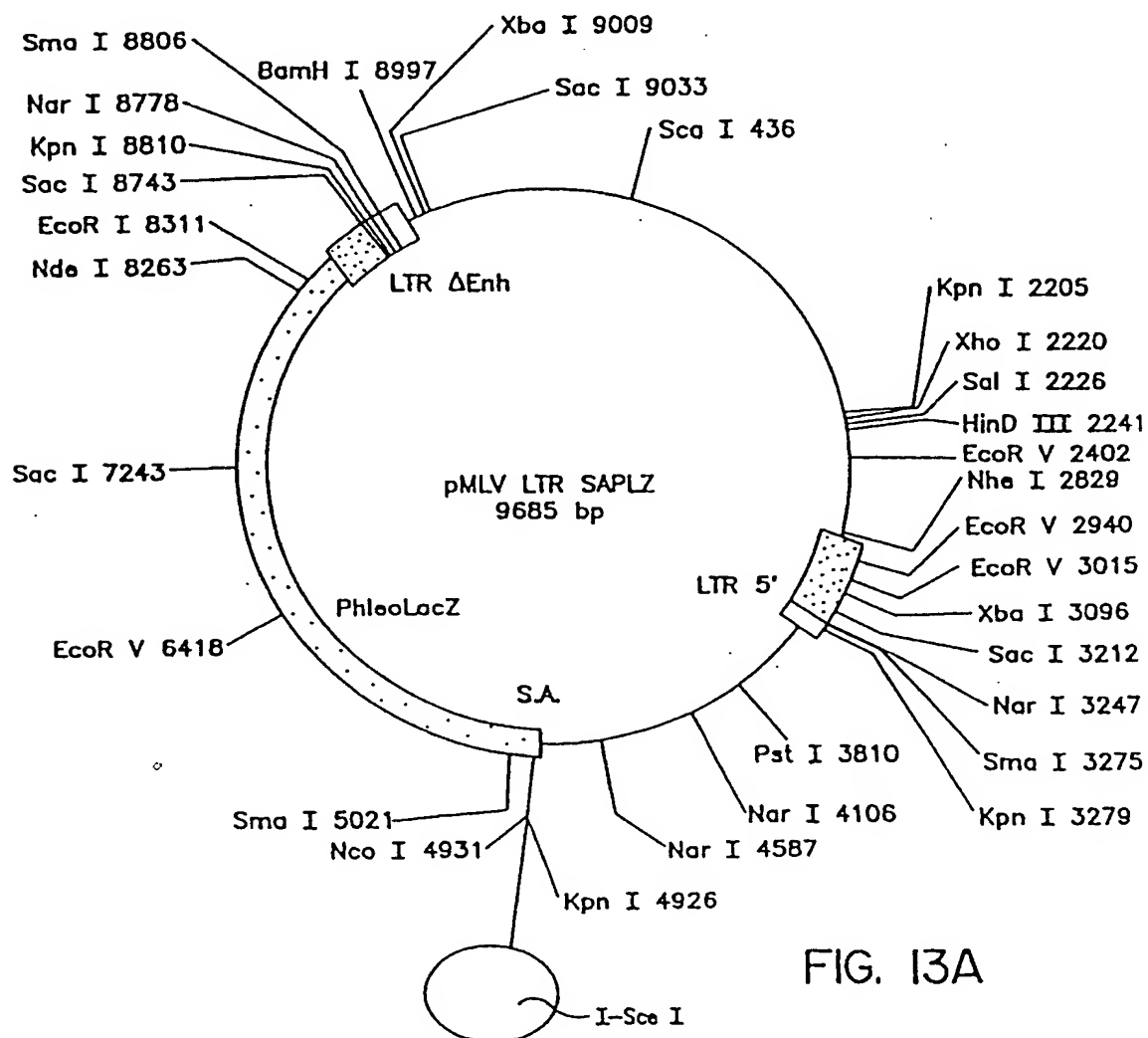


FIG. 13A

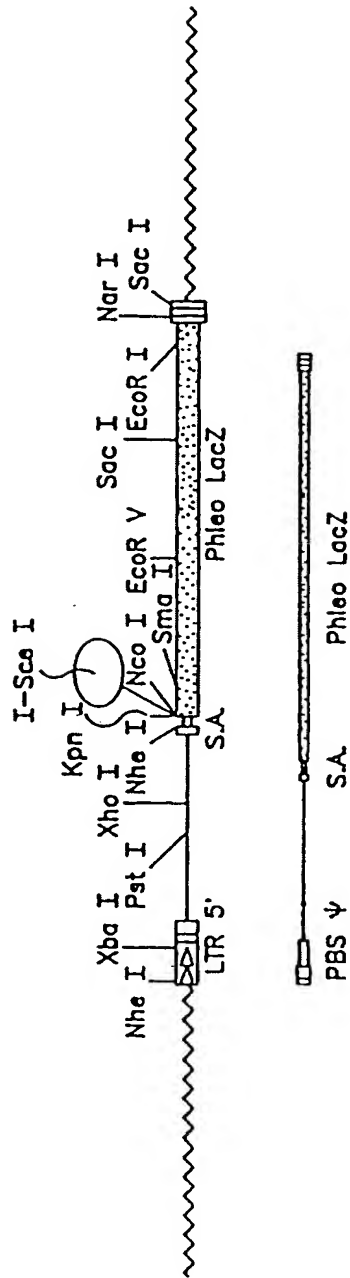


FIG. 13B

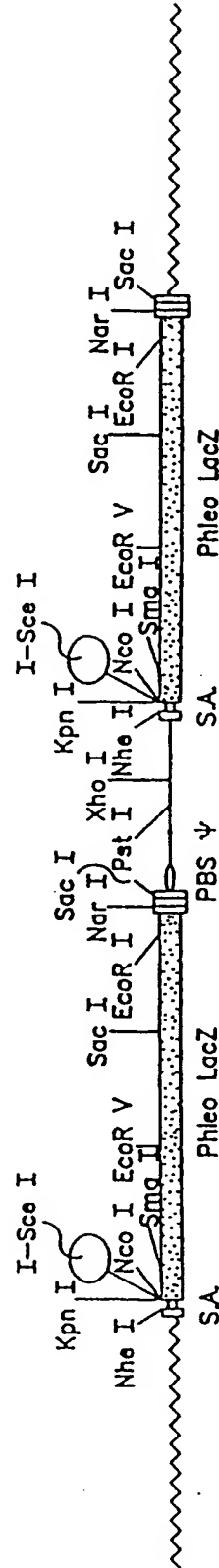
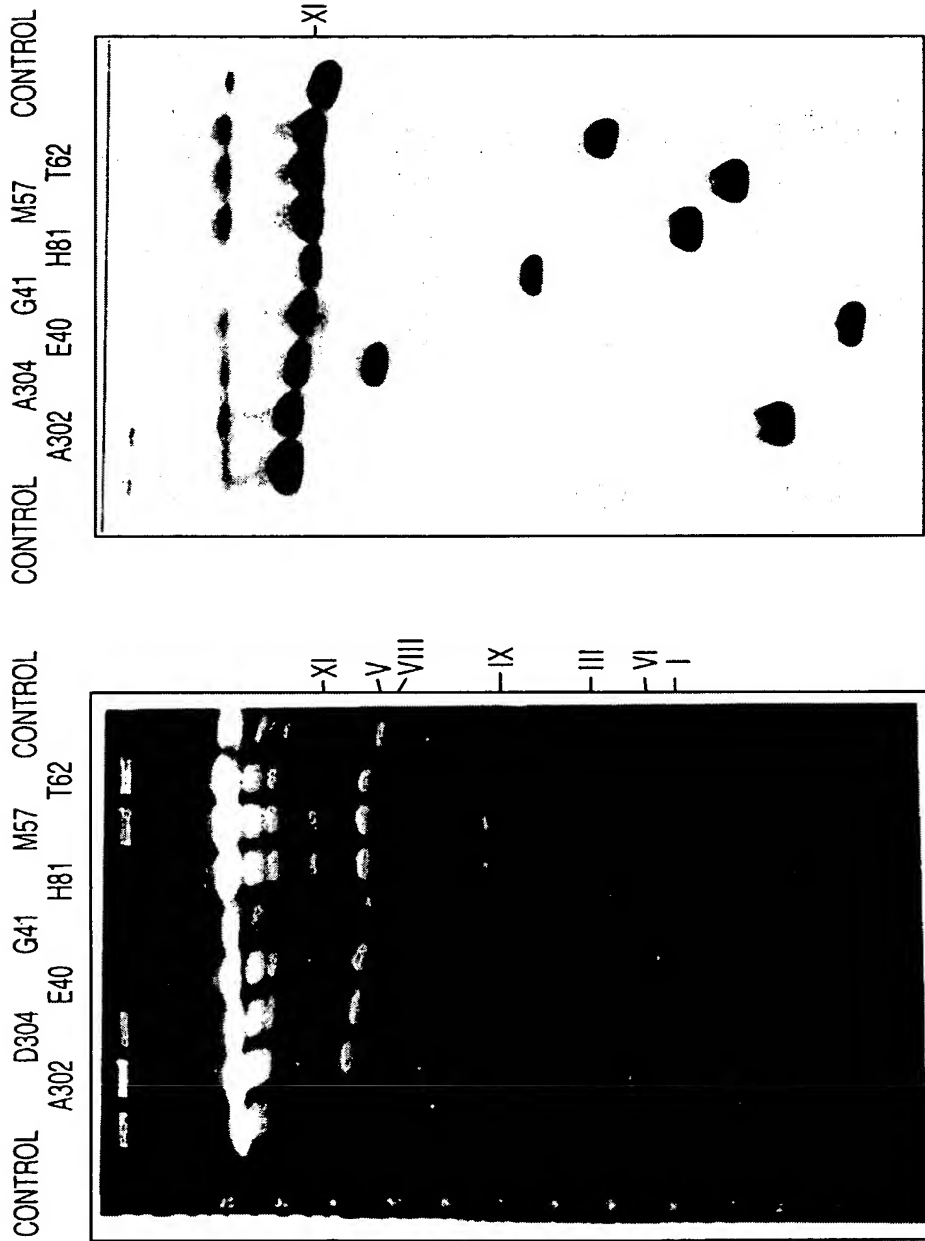


FIG. 13C



LEFT END PROBE  
COSMID pUKG 040

FIG. 14A

FIG. 14B



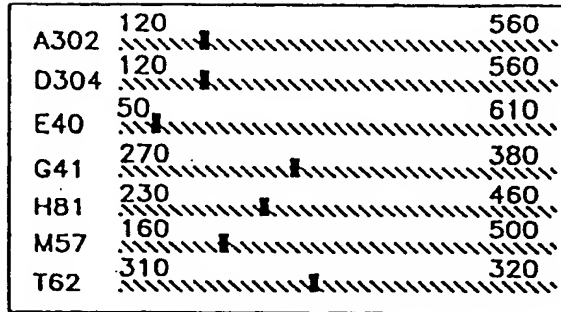


FIG. 15A

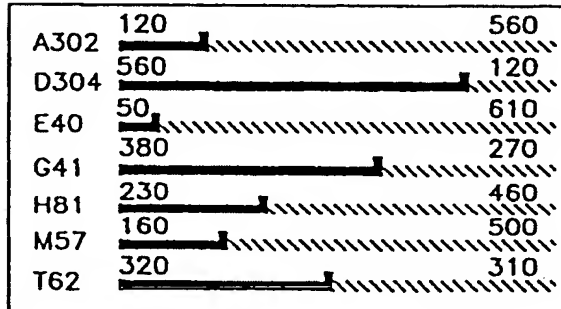


FIG. 15B

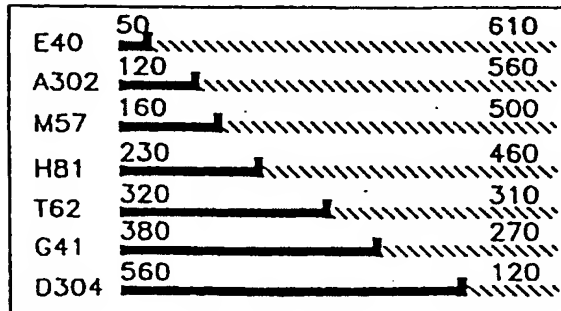


FIG. 15C

FIG. I5D

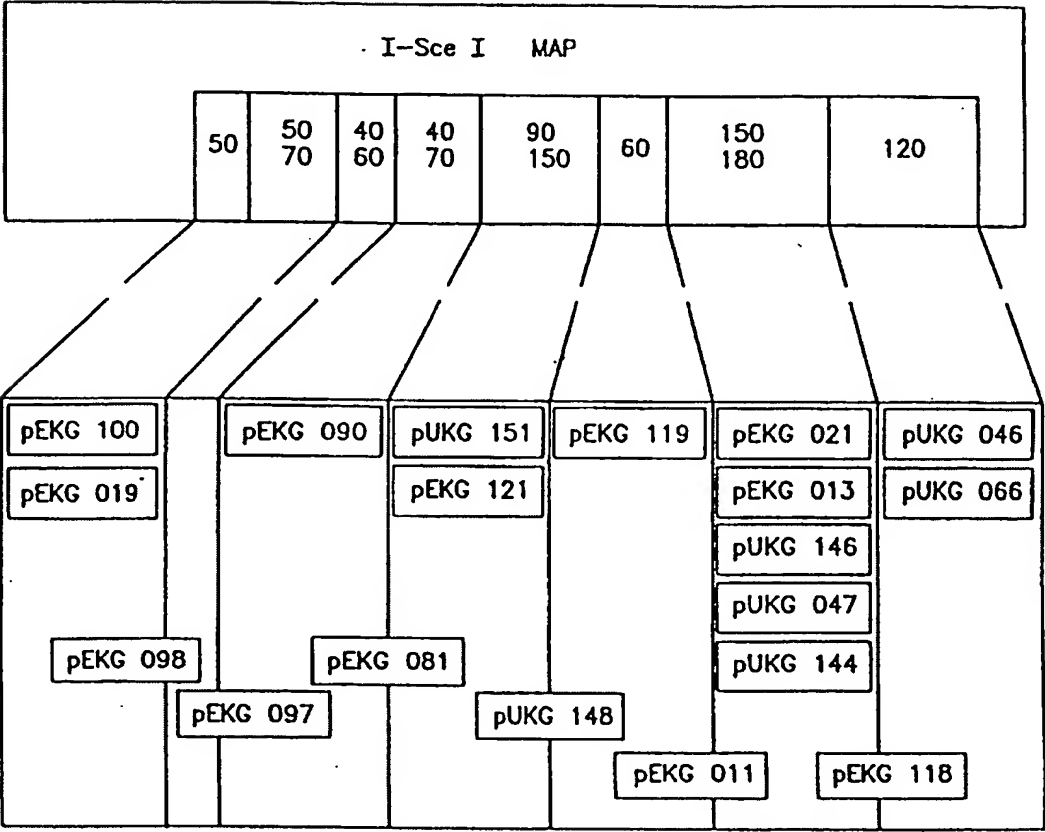
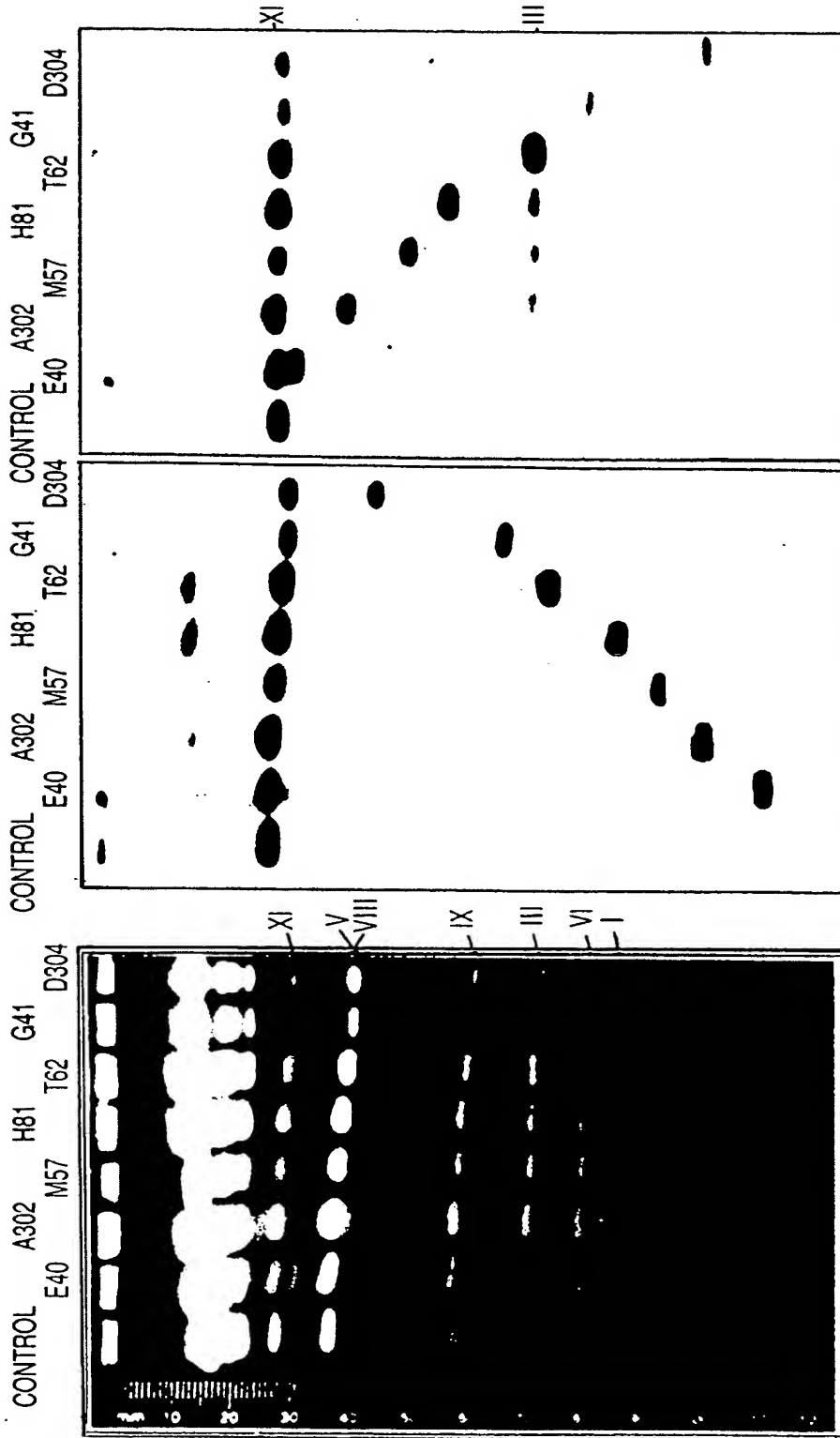


FIG. I5E



RIGHT END PROBE  
COSMID pUKG066

LEFT END PROBE  
COSMID pUKG040

FIG. 16C

FIG. 16B

FIG. 16A

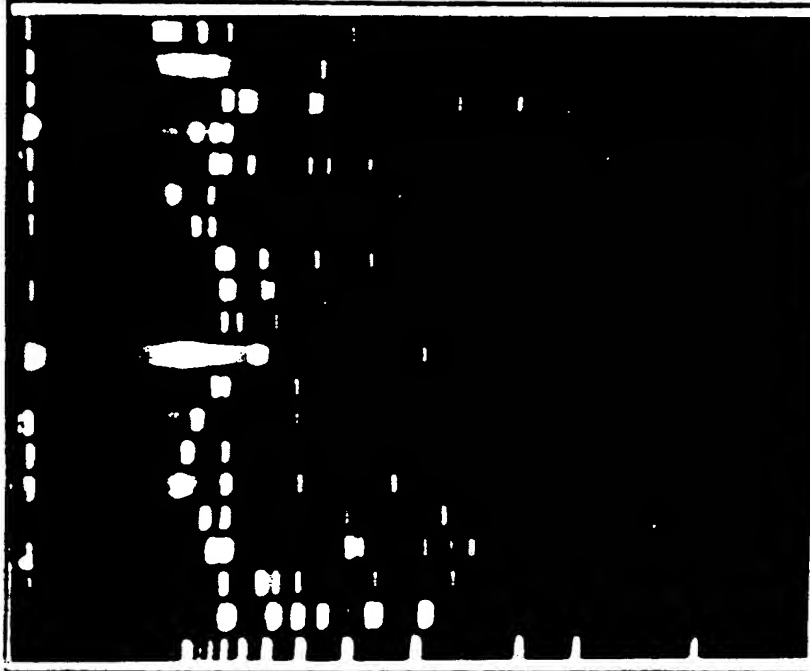


FIG. 17B

pEKG pEKG pEKG pEKG pEKG pEKG pEKG pEKG pEKG pEKG pEKG pEKG pEKG pEKG pEKG pEKG  
019 097 081 121 119 021 146 144 046  
pEKG pEKG pEKG pEKG pEKG pEKG pEKG pEKG pEKG pEKG pEKG pEKG pEKG pEKG pEKG pEKG  
100 098 090 151 148 011 047 013 118 066

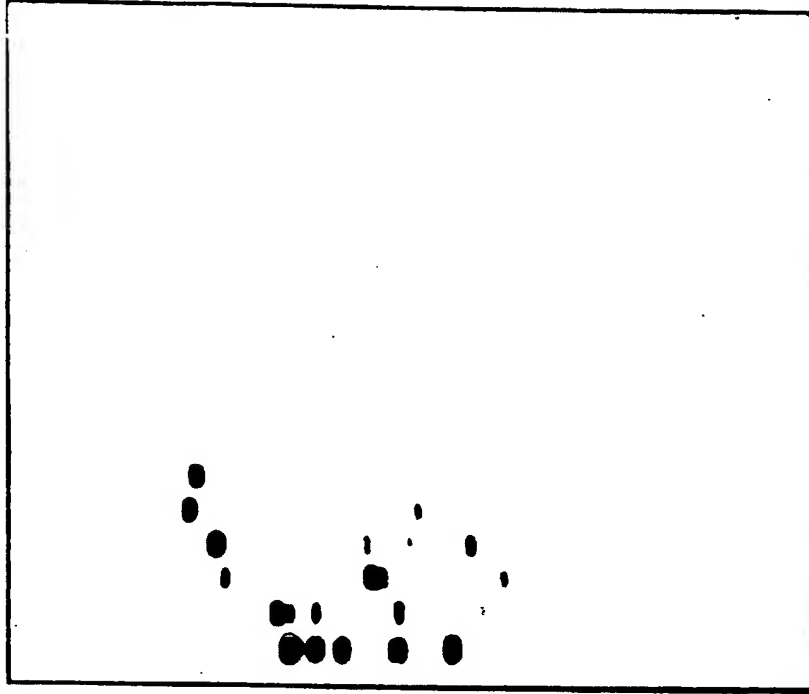


FIG. 17D

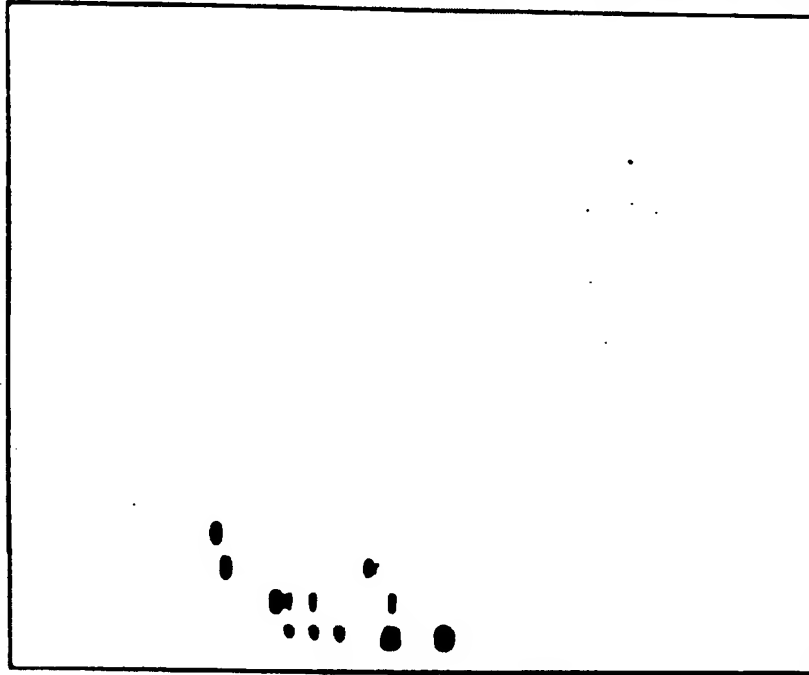


FIG. 17C

pEKG pEKG pEKG pEKG pEKG pEKG pEKG pEKG pEKG pEKG pEKG pEKG pEKG pEKG pEKG pEKG  
019 097 081 121 119 021 146 144 046  
pEKG pEKG pEKG pEKG pEKG pEKG pEKG pEKG pEKG pEKG pEKG pEKG pEKG pEKG pEKG pEKG  
100 098 090 151 148 011 047 013 118 066

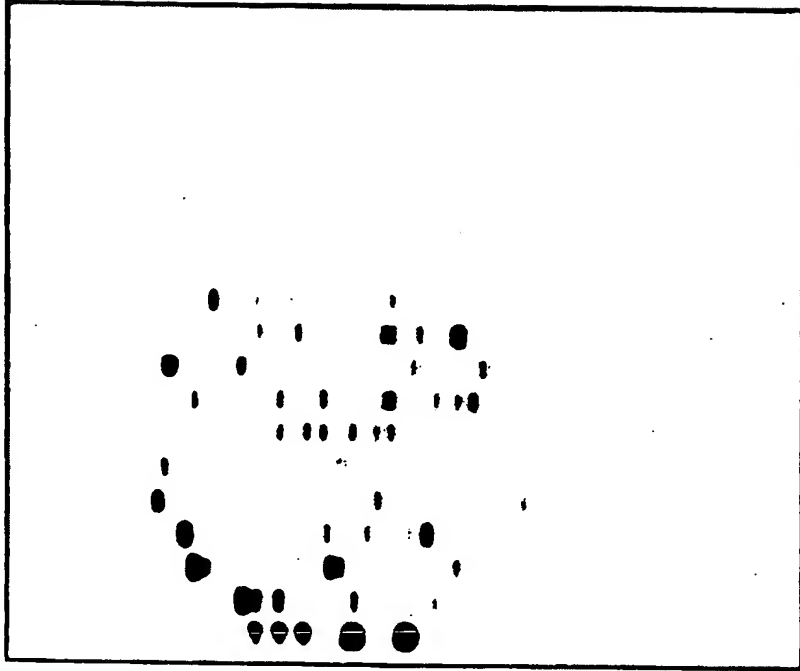


FIG. 17F

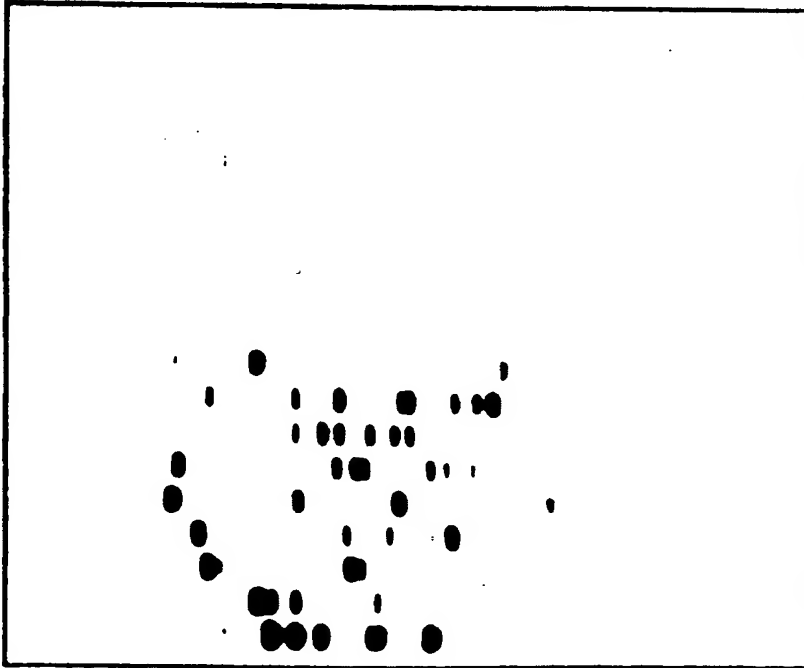


FIG. 17E

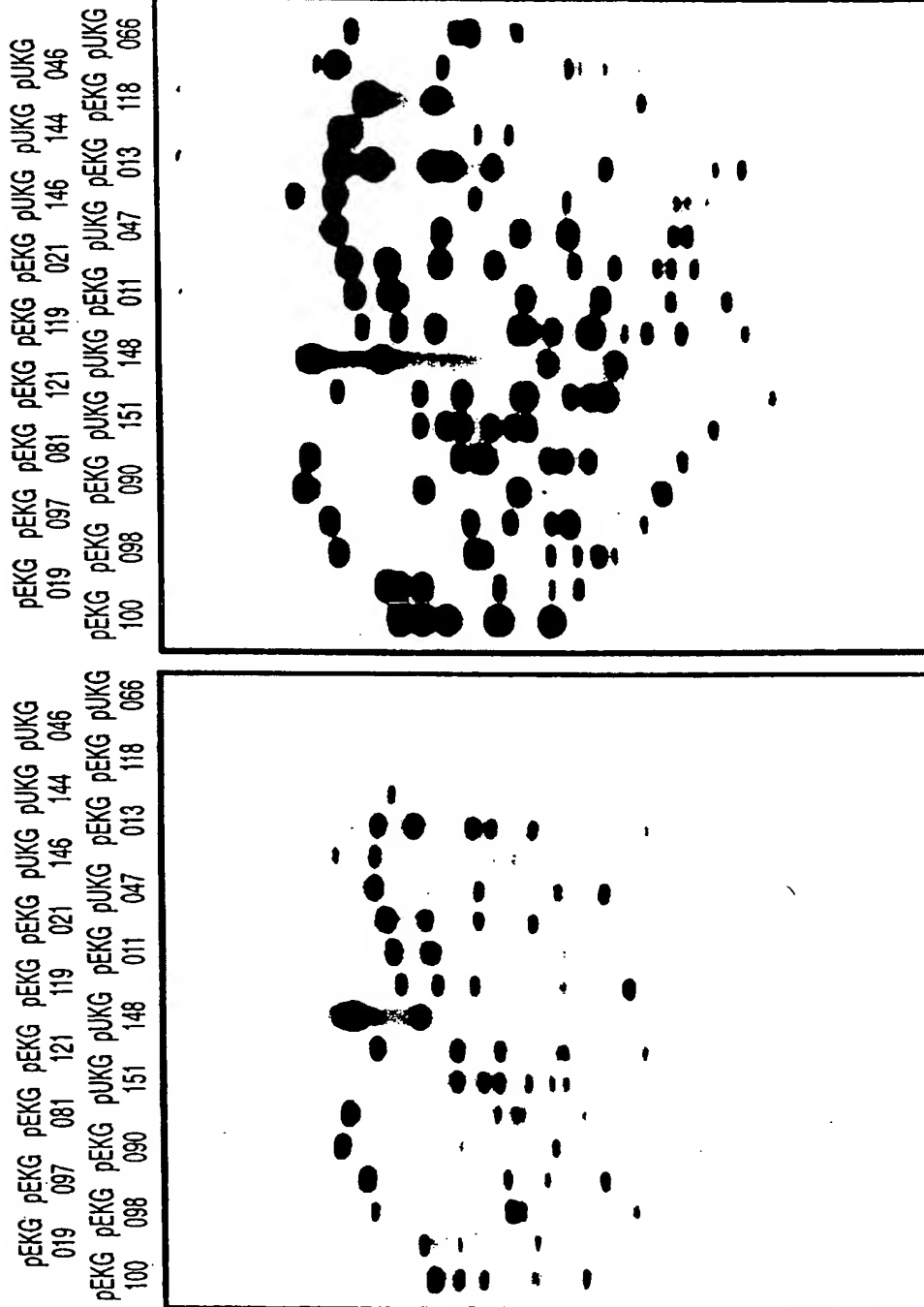


FIG. 17H

FIG. 17G

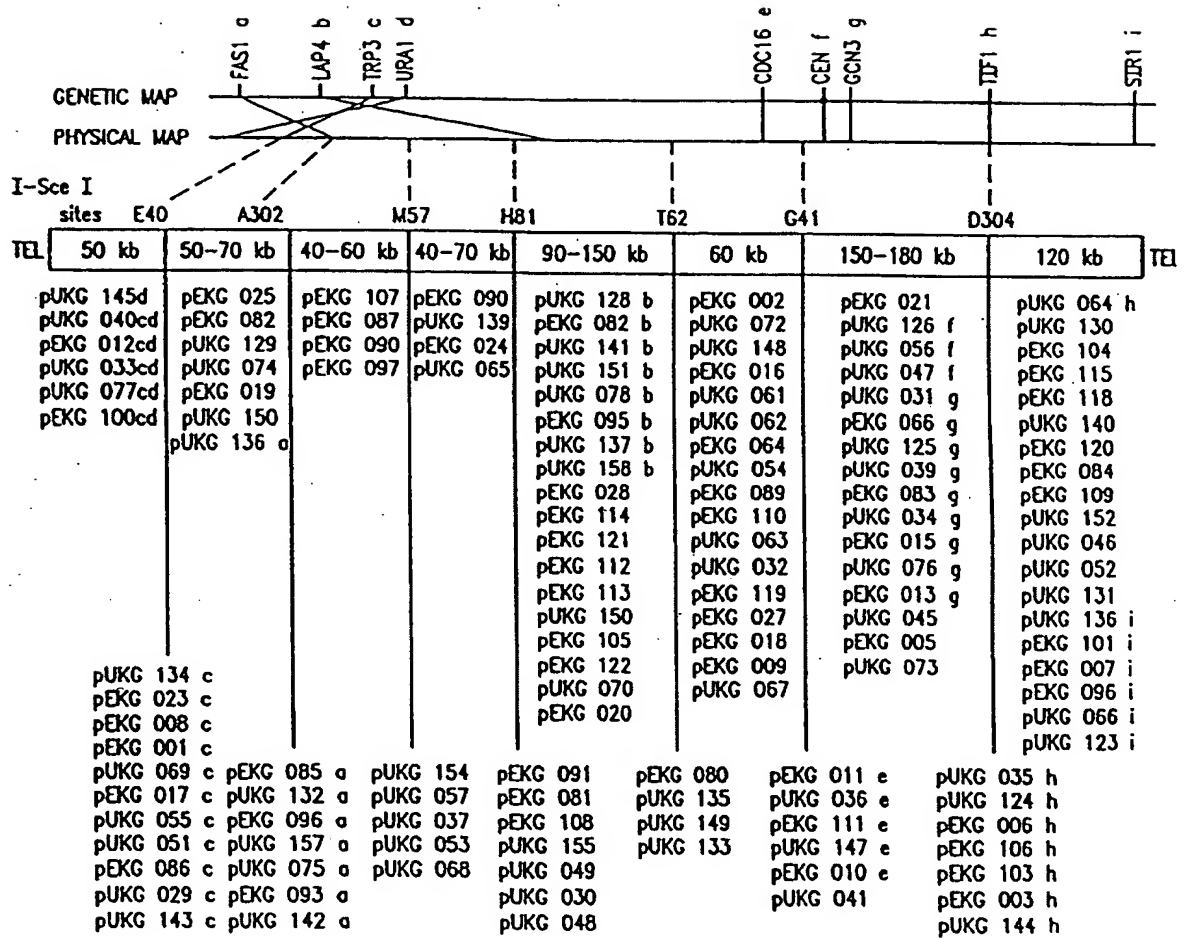


FIG. 18



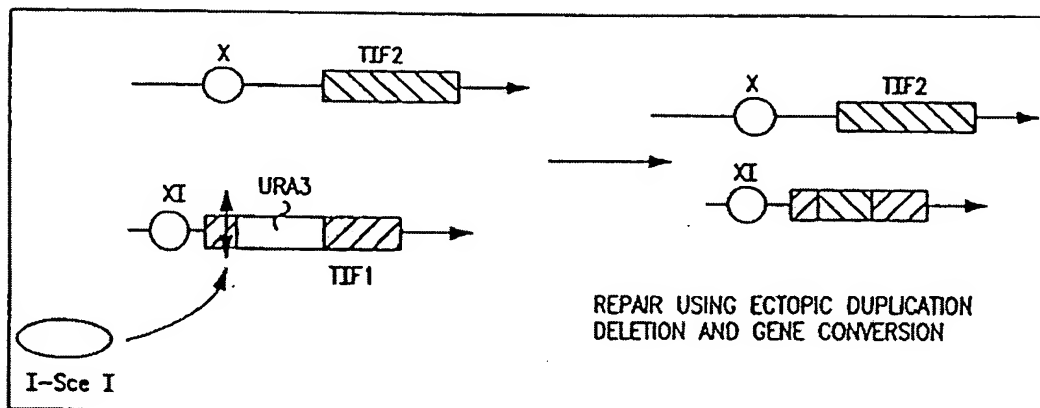


FIG. 19A

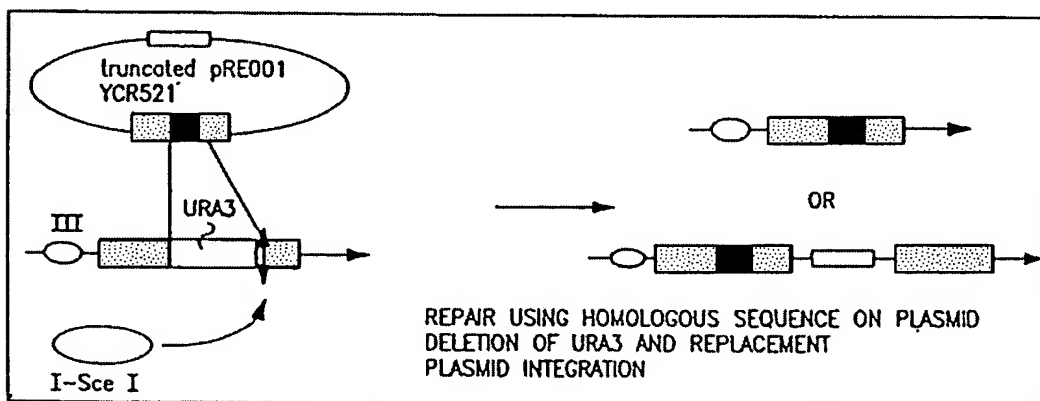
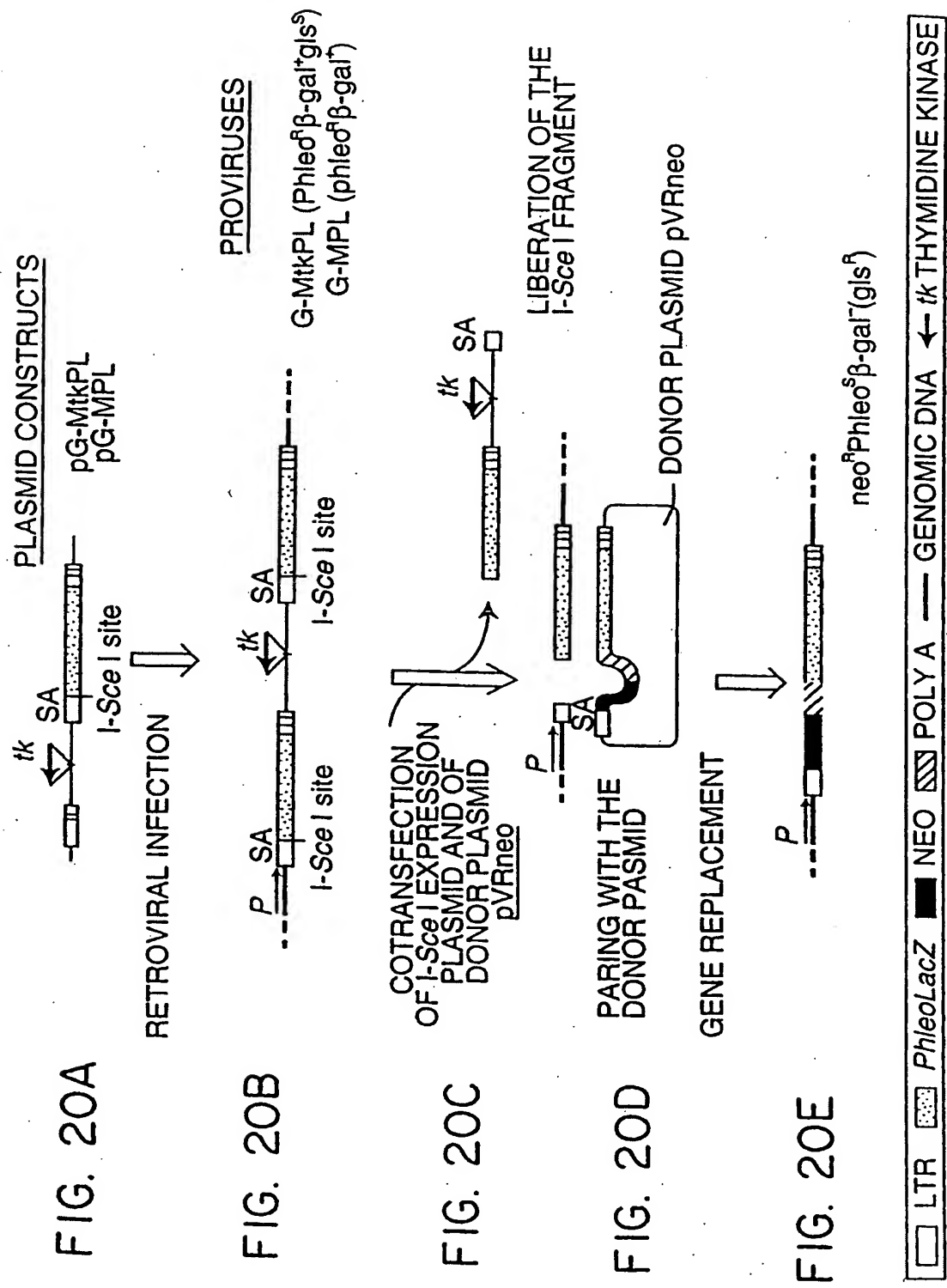


FIG. 19B



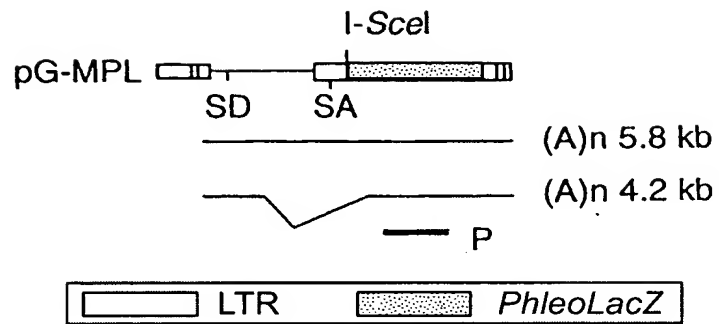


FIG. 2IA

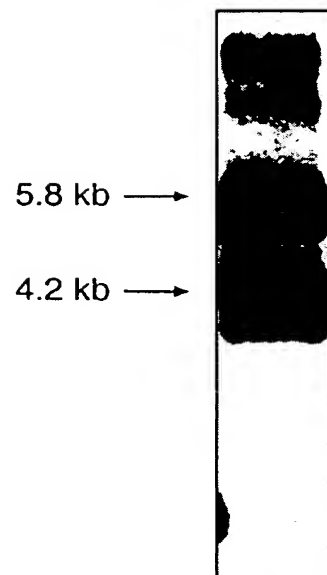


FIG. 21B

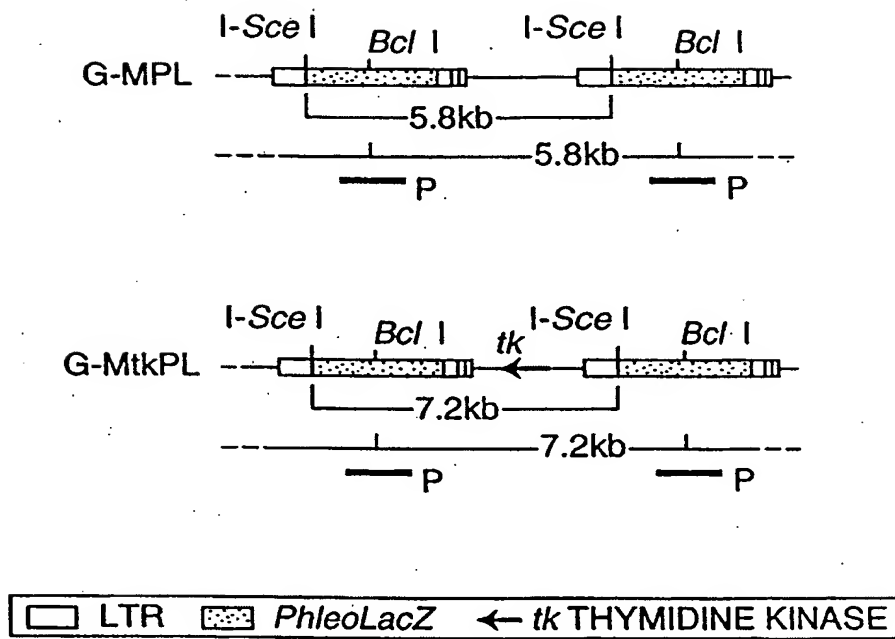


FIG. 22A

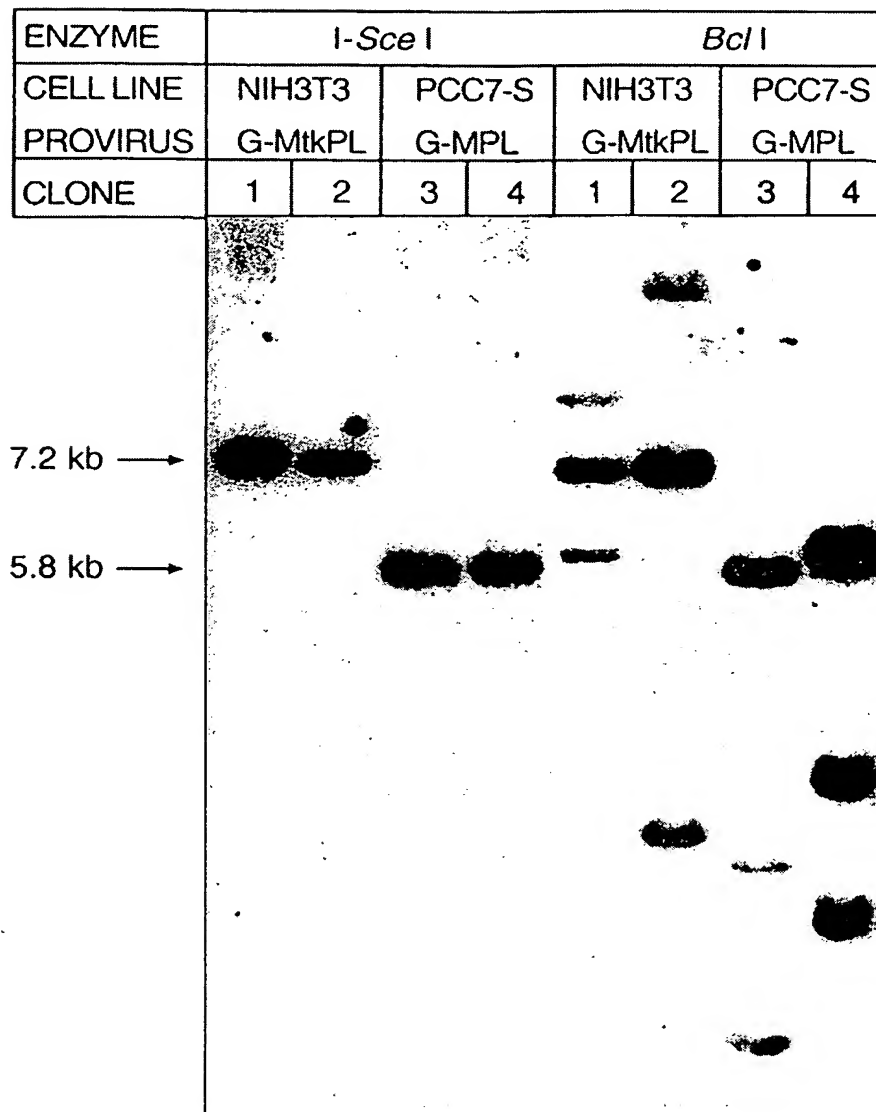


FIG. 22B

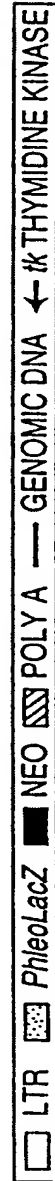
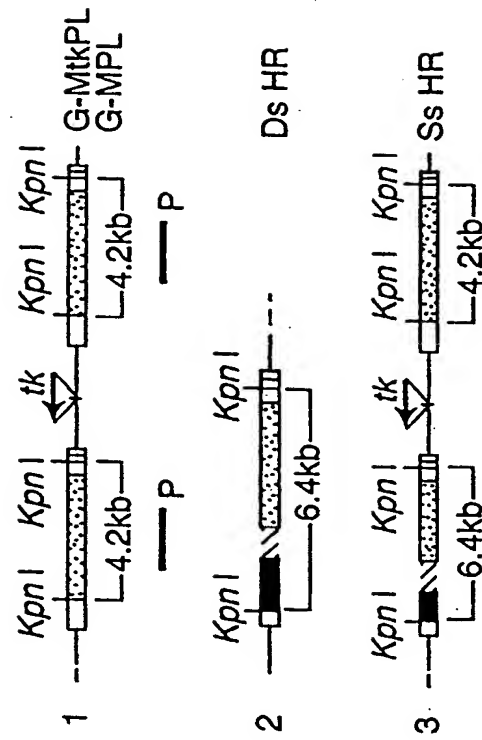


FIG. 23A

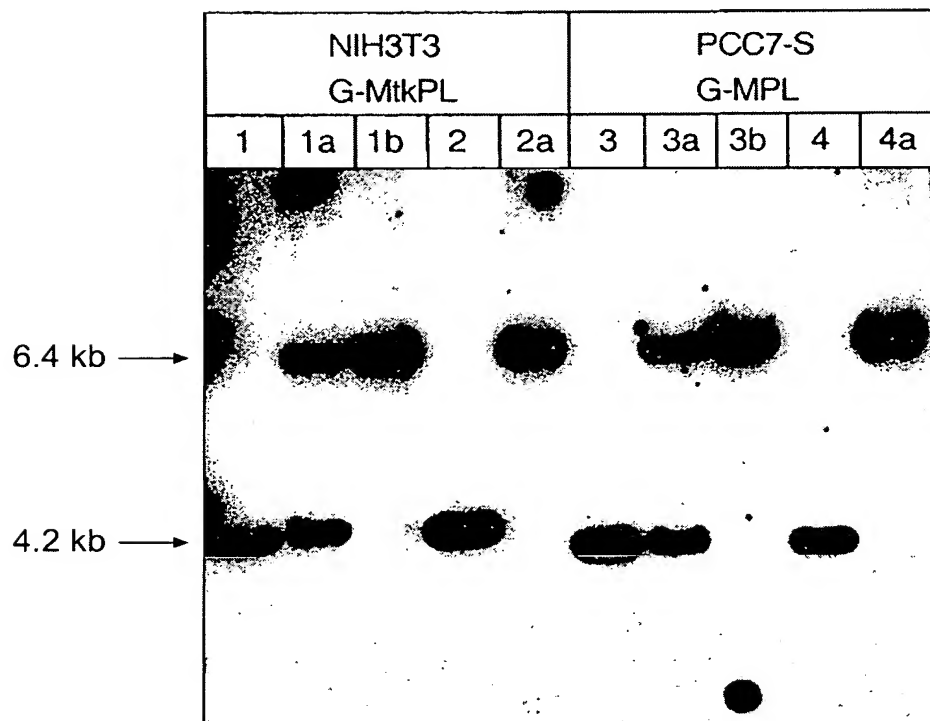


FIG. 23B



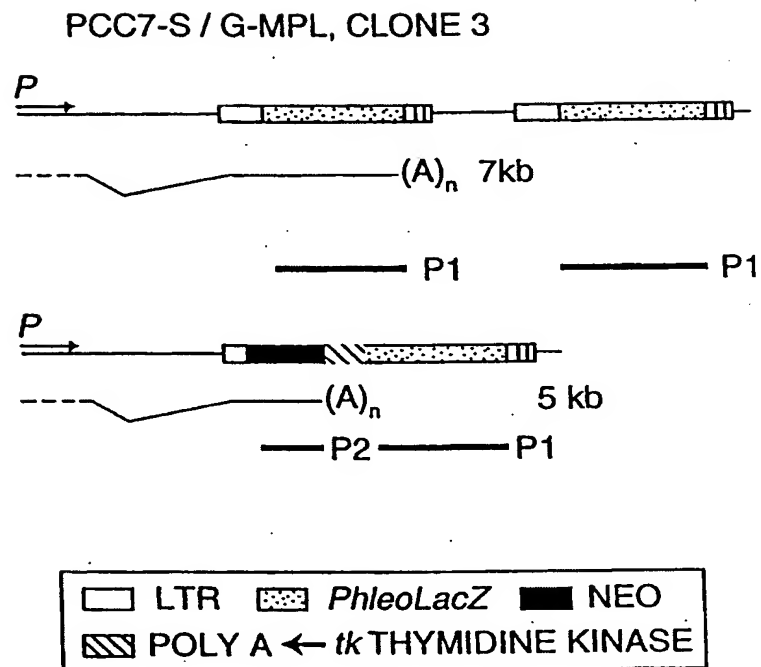


FIG. 24A

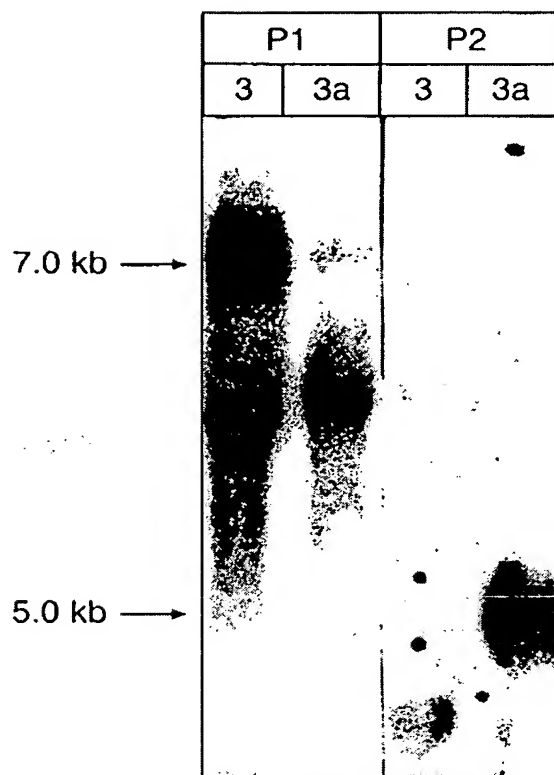
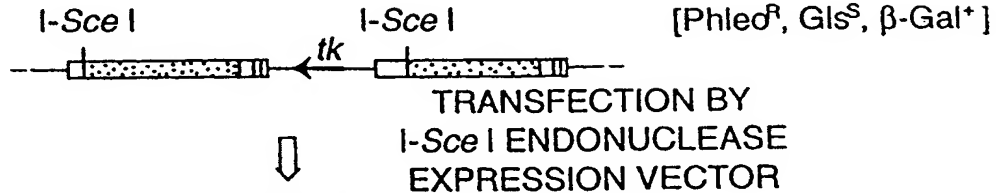


FIG. 24B

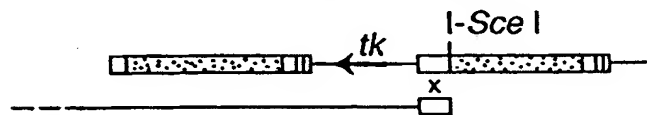
A. CHROMOSOMAL DNA  
CONTAINING PROVIRUS

PHENOTYPES

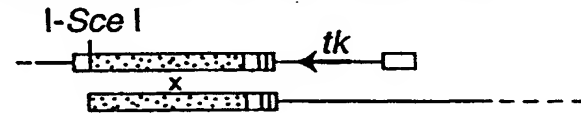


B. INTRA-CHROMOSOMAL  
RECOMBINATIONS EVENTS

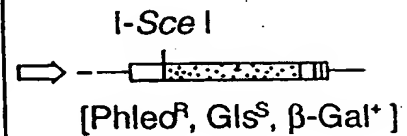
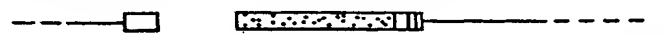
1. THE LEFT I-Sce I IS CUT.  
PAIRING AND RECOMBINATION



2. THE RIGHT I-Sce I IS CUT.  
PAIRING AND RECOMBINATION



3. BOTH I-Sce I SITES ARE CUT.  
RELIGATION BY END-JOINING



C. INTER-CHROMOSOMAL RECOMBINATION EVENT  
BOTH I-Sce I SITES ARE CUT. GAP REPAIR USING INTACT  
CHROMOSOME SEQUENCES

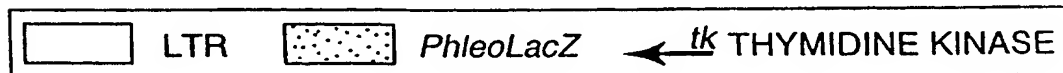
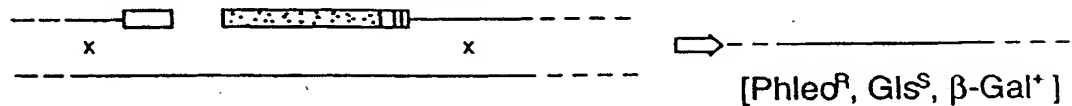


FIG. 25

A. PARENTAL DNA, G-MtkPL

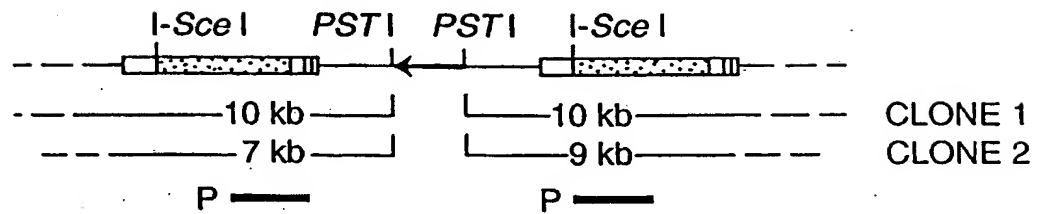


FIG. 26A

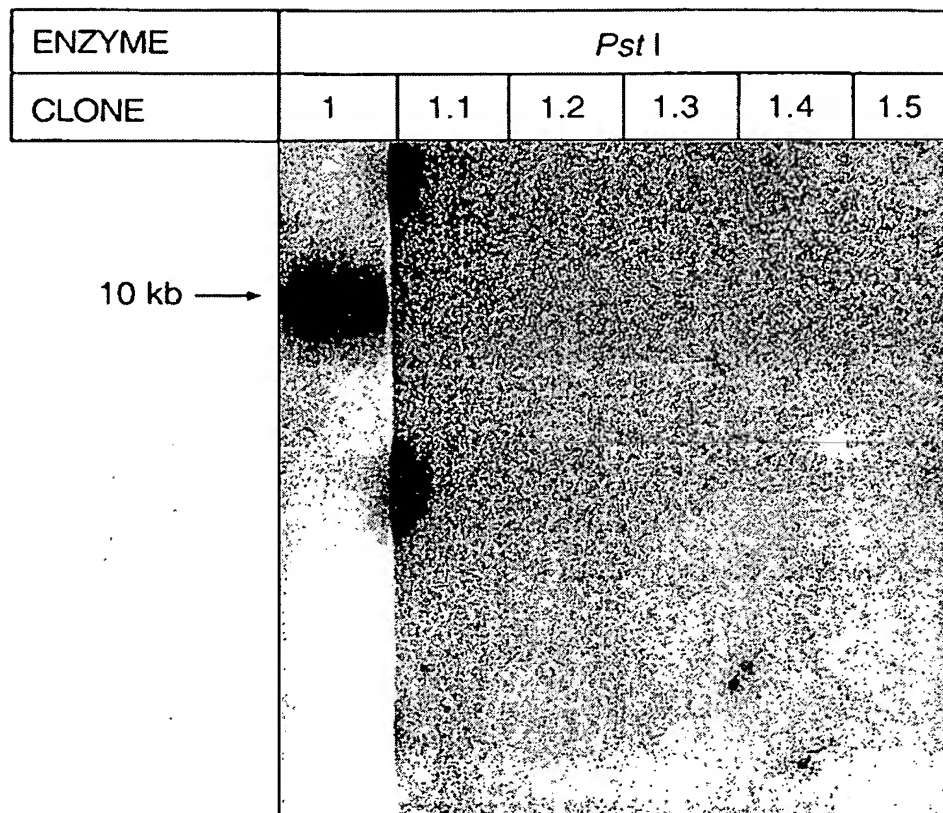


FIG. 26B

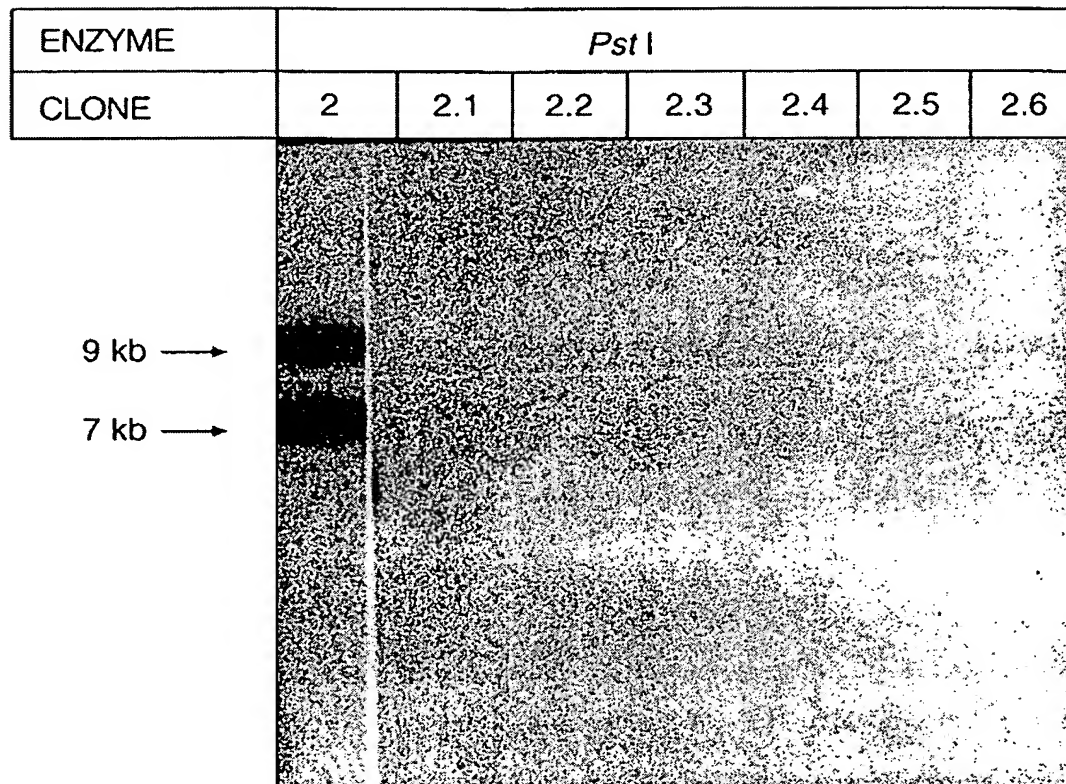
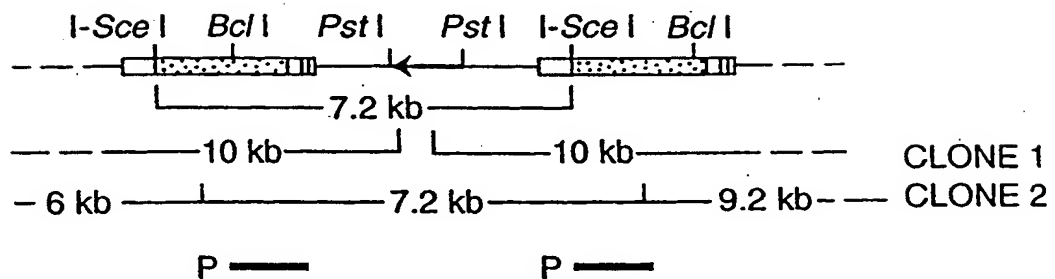


FIG. 26C

1. PARENTAL DNA, G-MtkPL



2. INTRA-MOLECULAR RECOMBINATION EVENT

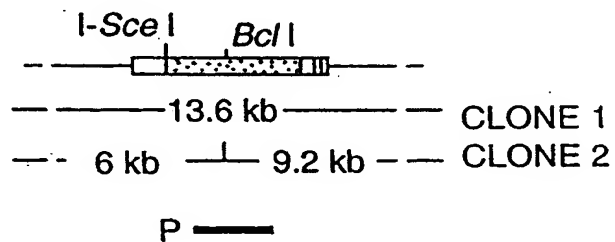


FIG. 27A

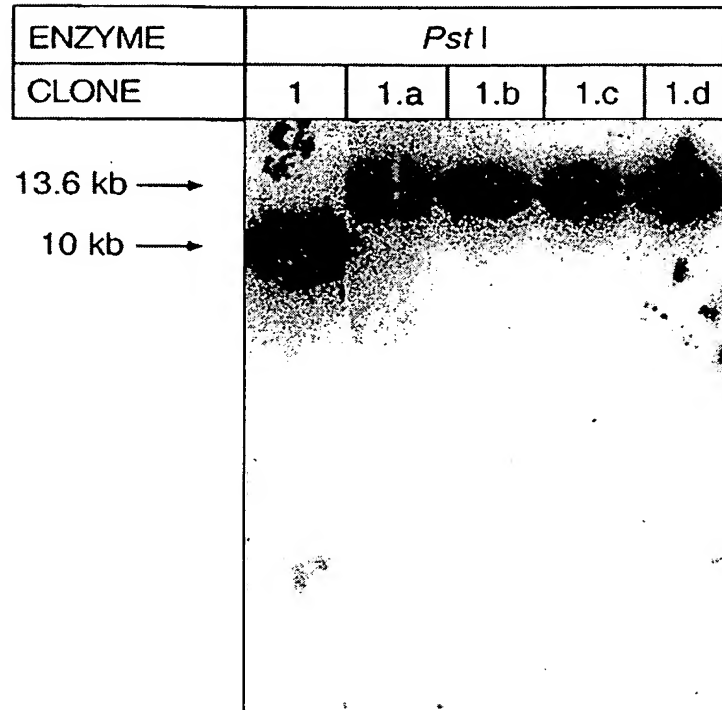


FIG. 27B



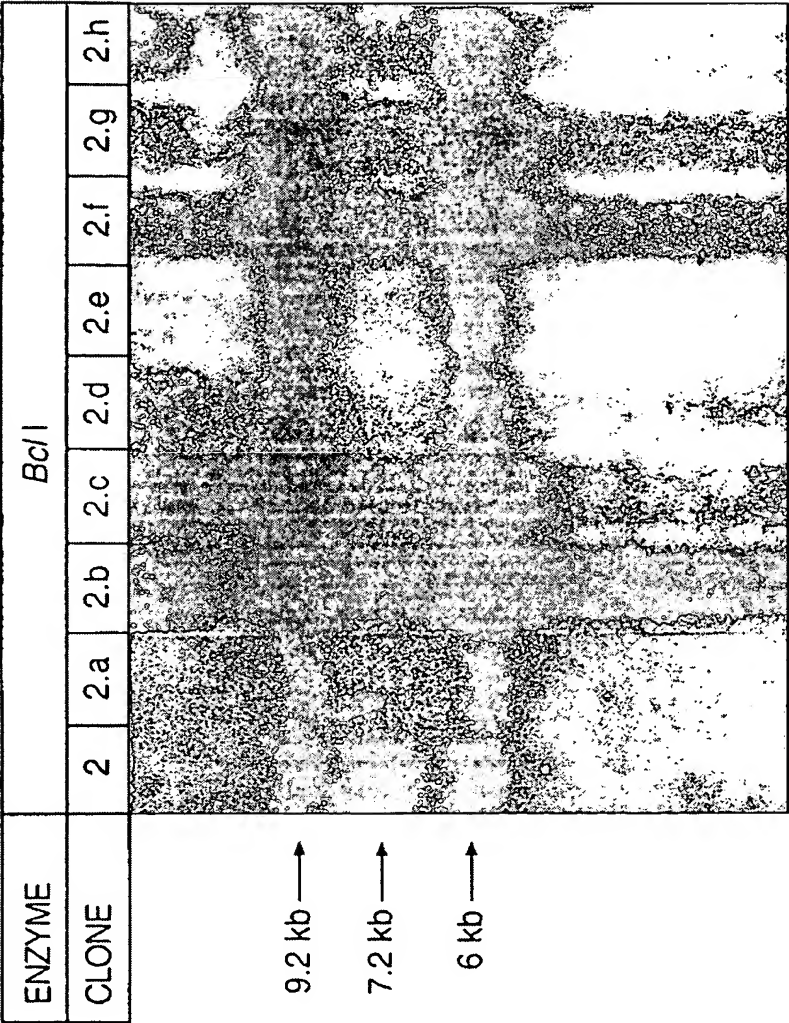
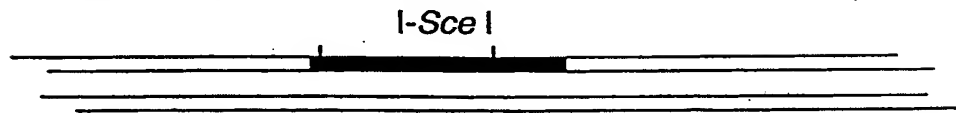


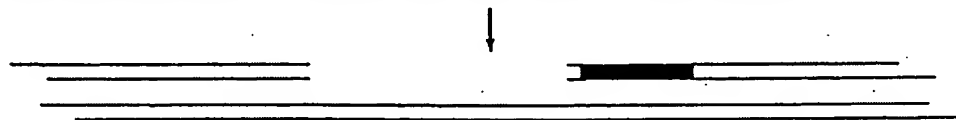
FIG. 27C

### LOSS OF HETEROZYGOSITY

INTEGRATION OF ARTIFICIAL SITE OR  
PRESENCE OF SPECIFIC SITE



EXPRESSION OF I-Sce I AND SPECIFIC CLEAVAGE



REPAIR OF THE DSB WITH THE OTHER CHROMATID

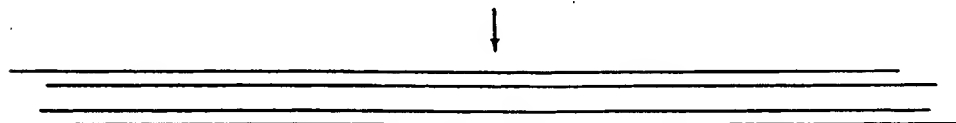


FIG. 28

### CONDITIONAL ACTIVATION (TANDEM REPEAT)

#### INTEGRATION OF ARTIFICIAL SITE BETWEEN TANDEM REPEATS



GENE X INACTIVE

#### EXPRESSION OF I-Sce I AND SPECIFIC CLEAVAGE



#### REPAIR OF THE DSB BY SINGLE STRAND ANNEALING

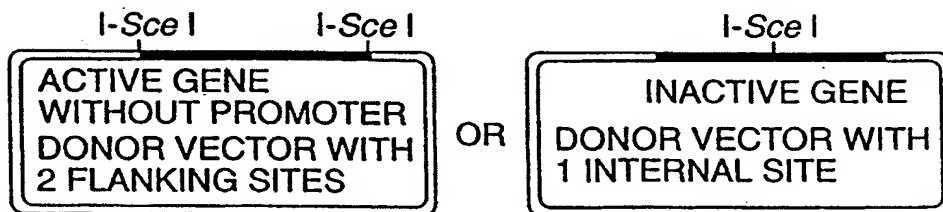


GENE X ACTIVE

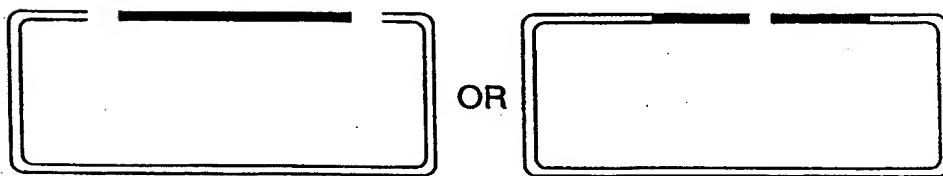
FIG. 29

## ONE STEP REARRANGEMENT

INTEGRATION OF ARTIFICIAL SITE OR  
PRESENCE OF SPECIFIC SITE



EXPRESSION OF I-Sce I ENZYME  
AND  
SPECIFIC CLEAVAGE OF THE DONOR PLASMID



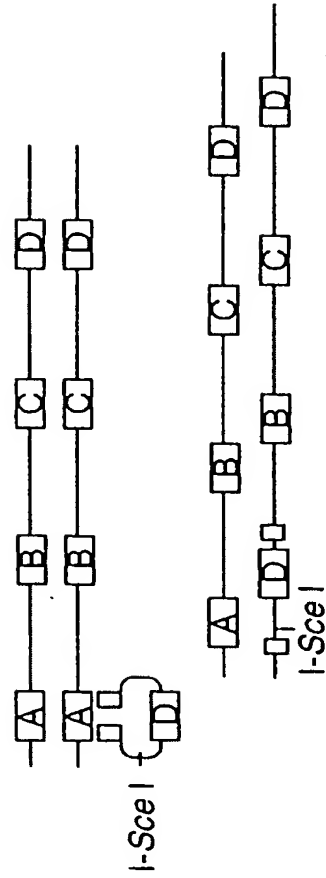
RECOMBINATION BETWEEN THE CHROMOSOME AND PLASMID



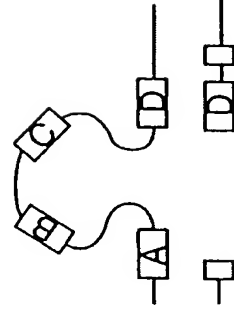
FIG. 30

## DUPLICATION OF A LOCUS

### 1. INSERTION OF I-Sce I SITE BY CLASSICAL GENE REPLACEMENT



### 2. SPECIFIC CLEAVAGE BY I-Sce I ENZYME AND REPAIR OF THE BREAK BY HOMOLOGOUS SEQUENCES



### 3. DUPLICATION OF THE TOTALITY OF THE LOCUS

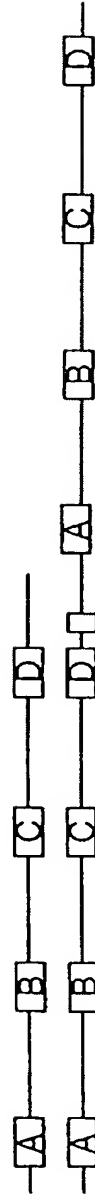
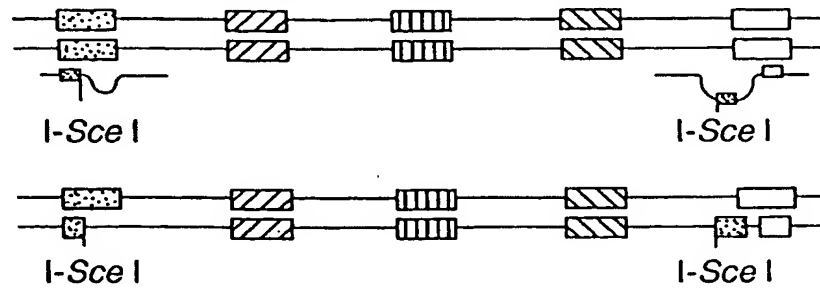


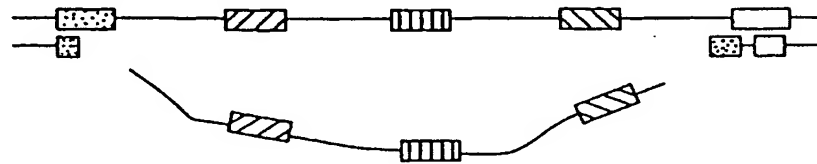
FIG. 31

## DELETION OF A LOCUS

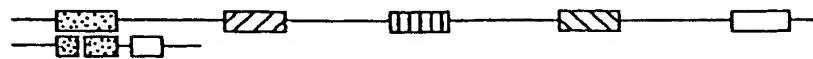
### 1 INSERTION OF TWO I-Sce I SITES FLANKING THE LOCUS



### 2 EXPRESSION OF THE ENZYME AND CLEAVAGE



### 3 RECOMBINATION BETWEEN THE TWO ENDS



### 4 DELETION OF THE LOCUS

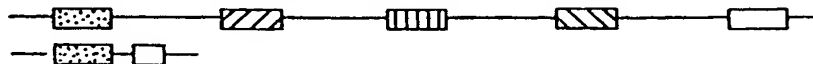


FIG. 32

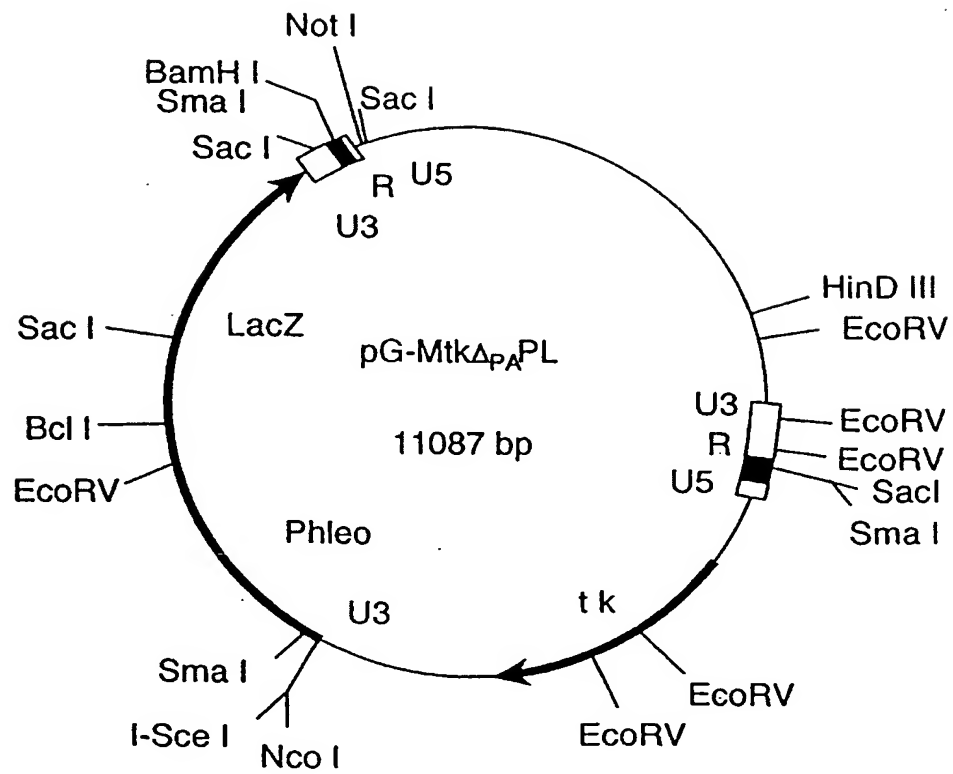


FIG. 33